EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Materials and Experimental Methods

Rats: Female Lewis rats, six weeks old, were purchased from Harlan (Israel) and maintained under SPF conditions in an animal facility.

Peptide antigens: Myelin Basic Protein (MBP) p68-86, Y G S L P Q K S Q R S Q D E N P V (SEQ ID NO:1), was synthesized on a MilliGen 9050 peptide synthesizer by standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by high performance liquid chromatography. Structure was confirmed by amino acid analysis and mass spectroscopy. Only peptides that were greater than 95 % pure were used in our study.

Immunizations and induction of active disease: Rats were immunized subcutaneously in the hind foot pads with 0.1 ml of MBP epitope 68-86 (p68-86) dissolved in PBS (1.5 mg/ml) and emulsified with an equal volume of CFA (incomplete Freund's adjuvant supplemented with 4 mg/ml heat-killed *Mycobacterium tuberculosis* H37Ra in oil (Difco laboratories, Inc., Detroit, MI). Rats were then monitored for clinical signs daily by an observer blind to the treatment protocol. EAE was scored as follows: 0, clinically normal; 1, flaccid tail; 2, hind limb paralysis; 3, front and hind limb paralysis.

Induction of transferred EAE: EAE was induced by immunizing Lewis rats (intraperitoneally) with 10^8 activated spleen cells from EAE donors obtained as follows: Nine days after induction of active EAE, splenic cells were cultured (12 x 10^6/ml) at 37 °C in humidified air containing 7.5 % CO_2 for two days in stimulation medium that includes Dulbecco's modified Eagle's medium (Gibco) supplemented with 2-mercaptoethanol (5 x 10^{-5} M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 μg/ml), streptomycin (100 μg/ml), 1 % syngeneic serum and 20-30 μg/ml of the immunizing epitope. Then, cells were separated on a Ficoll gradient (Sigma), resuspended in PBS and injected to naive recipients.

Antigen-specific T cell proliferation assays: Lewis rats were immunized with MBP p68-86/CFA as described above. Nine to ten days later spleen cells were suspended in stimulation medium and cultured in U-

shape 96-well microculture plates (2 x 10^5 cells/well) for 72 hours, at 37 °C in humidified air containing 7.5 % CO_2. Each well was pulsed with 2 μCi of [^{3}H]-Thymidine (specific activity 10 Ci/mmol) for the final six hours. The cultures were then harvested on fiberglass filters and the proliferative response expressed as CPM ± S.E. or as stimulation index (SI) (mean CPM of test cultures divided by mean CPM of control cultures).

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis: RT-PCR analysis, verified by Southern blotting, was utilized on brain samples according to the protocol described elsewhere with some modifications (39). Rats were euthanized by CO_2 narcosis. Brain samples containing mainly the midbrain and brain stem were obtained after perfusion of the rat with 160-180 ml of ice-cold phosphate buffered saline (PBS) injected into the left ventricle following an incision in the right atrium. Each sample was homogenized. Total RNA was extracted using the Tri-Zol procedure (Gibco BRL) according to the manufacturer's protocol. mRNA was then isolated using a mRNA isolation kit (#1741985, Boheringer Mannheim, Germany), and reverse transcribed into first strand cDNA as described in detail elsewhere (39). First strand cDNA was then subjected to 35 cycles of PCR amplification using specific oligonucleotide primers to rat IGIF and IFN-γ which were designed based on the published sequence of each cytokine (NCBI accession number for rat IGIF - U77777; and for rat IFN-γ - M29315) as follows (Table 1):

TABLE 1

Name/Function	Sequence	SEQ ID NO:
Rat IGIF sense	5'-ATGGCTGCCATGTCAGAAGAAG-3'	2
Rat IGIF antisense	5'-CTAACTTTGATGTAAGTTAGTAAGA-3'	3
Rat IFN-γ sense	5'-TACTGCCAAGGCACACTCATTGAA-3'	4
Rat IFN-γ antisense	5'-CGCTTCCTTAGGCTAGATTCTGG-3'	5
Rat β-actin sense	5'-CATCGTGGGCCGCTCTAGGCA-3' *	6
Rat β-actin antisense	5'-CCGGCCAGCCAAGTCCAGACG-3' *	7

Sequence according to Reference 39.

Experimental conditions were calibrated so RT-PCR amplifications fall on the linear part of the titration curve. The cycle profile was: denaturation at 95 °C for 60 seconds, annealing at 55 °C for 60 seconds, and

elongation at 72 °C for 60 seconds. Amplified products were subjected to electrophoresis, transferred to a nylon membranes (MagnaGraph nylon transfer membrane, msi, Westborough, MA), fixed with ultraviolet light (120 mjoules) and hybridized with 10^6 cpm/ml of a ^{32}P labeled DNA fragments encoding the full length PCR product of IGIF and of β-actin (random priming: Amersham, Arlington Heights, IL). PCR products were used as probes only after each PCR product was cloned and its sequence was verified as described below. Southern bolt images were objectively assessed using an FujiFilm Thermal System FTI-500 (FujiFilm, Japan).

Cloning and sequencing of PCR products: Each of the amplified PCR products described above was cloned into a pUC57/T vector (T-cloning Kit #K1212, MBI Fermentas, Lithuania) and transformed to *E. coli* according to the manufacturer's protocol. Each clone was then sequenced (Sequenase version 2, USB, Cleveland, Ohio) according to the manufacturer's protocol.

Production and purification of recombinant proteins. After sequence verification, PCR products were recloned into a PQE expression vector (PQE-30, PQE-31 or PQE-32 according the correct open reading frame) and expressed in E. Coli (Qaigen, Hilden, GmbH) and then purified by an NI-NTA-super flow affinity purification of 6 x His proteins (Qaigen). Each recombinant protein sequence has been verified (N -terminus).

Production and purification of Rabbit anti-rat IGIF IgG: Rabbit anti-rat IGIF antibodies were generated as described (40) and IgG fraction was purified using a HiTrap protein G kit (Pharmacia, Piscataway, NJ, Kit #17-040-01). Antibody titer was determined by a direct ELISA assay: ELISA plates (Nunc, Denmark) were coated with recombinant rat IGIF(50 ng/well). Rabbit anti-rat IGIF (IgG fraction) was added in serial dilutions from 2^8 to 2^{30}. Goat anti-rabbit IgG alkaline phosphatase conjugated antibodies (Sigma) were used as a labeled antibody. p-Nitrophenyl Phosphate(p-NPP) (Sigma) was used as a soluble alkaline phosphatase substrate. Results of triplicates were calculated as $\log_2$ antibody titer ± SE. The purified anti-rat IGIF IgG titer was 18±0.4.

Cytokine determination: Spleen cells from EAE donors were stimulated *in vitro* (10^7 cells/ml) in 24 well plates (Nunc) with 100 μM p68-86. Spleen cells from naive donors were cultured (10^7 cells/ml, 24 well plates) with 2 μg/ml Con A (Sigma). After 72 hours of stimulation, supernatants were assayed by semi-ELISA kits, that include antibody pairs and recombinant rat cytokines, as follows: IFN-γ, rabbit anti-rat IFN-γ